Phenolic characterization of Italian cultivar of Phaseolus vulgaris beans

Phenolic characterization of Italian cultivar of *Phaseolus vulgaris* beans

Pamela Vignolini, Maria Angela Falvino, Patrizia Pinelli, Annalisa Romani

Department of Pharmaceutical Sciences, University of Florence, Sesto Fiorentino, Via Ugo Schiff 6, 50019, *Florence*

Abstract. Flavonols (kaempferol and quercetin derivatives), hydroxycinnamic acid (caffeic, ferulic and p-coumaric acid derivatives), and anthocyanins (cyanidin and pelargonidin glycosides) in one pigmented variety (red San Michele), have been characterized and quantified in four bean varieties (Sarconi, IGP) referring to hulls and whole grain flours extracts, by HPLC/DAD/MS analysis. This allowed to compare, within a single variety, the quali-quantitative data of hull extracts and whole grain flours in order to identify their distribution in different parts of seed, and to assess the differences related to variability on annual production in 2007 and 2008 years. The results showed that the second year, if indeed sacrificed in production due to prolonged drought, could be defined as an excellent year for polyphenol production (0.303-3.742 mg/g for whole grain flours and 0.975-8.15 mg/g for seed-coat extracts). The optimization of analytical methods for the assessment of antiradical and antioxidant activities allowed to define the biological and functional properties of each analyzed variety. The antioxidant capacity evaluated by DPPH test has been measured in the all samples, the EC_{50} data range from 2.78 mg/mg (San Michele, Belisario) to 16.93mg/mg (Riso Bianco, De Rosa).

Introduction. Since the second half of the twentieth century, many species of agricultural interest, for example beans, once widely cultivated for human food, have undergone a gradual contraction of the spread. The causes which could contribute to lower use of these crops are the following: the evolution of the welfare state, the change in eating habits, lack of standards for the cultural practices, globalization of markets and homologation of productions. Moreover, the loss of genetic diversity may also contribute directly to a flattening of the culture, with a gradual disappearance of traditions and customs. Beans are largely consumed in view of their high protein content and recent studies have pointed out that they supply the diet with complex carbohydrates, soluble fibres, essential vitamins and metals, as well as polyphenols [*1,2*], such as flavonoids, isoflavones and lignans [*3*]. Polyphenols have attracted increasing interest for their anticarcinogenic properties and play an important role in contrasting oxidative stress, which is defined as the imbalance between oxidants and antioxidants, identified as the cause of ageing and various diseases in humans [*4,5*]. The aims of this investigation was to characterize four bean varieties (Sarconi IGP) (Riso Bianco, red San Michele, Tabacchino, Verdolino) produced in two different farms, located in distal areas of the Val D'Agri (Potenza) for two production years (2007-2008). The purpose of the following work has been the optimization of HPLC/DAD/MS methods, in order to make the quali-quantitative characterization of polyphenols in each of the four bean varieties, referring to hulls and whole grain flour extracts.

Material and Methods. Dry seeds of four landraces of *Phaseolus vulgaris* L. (San Michele (SM), Tabacchino (T), Verdolino (V) and Riso Bianco (RB)) were collected in two different fields (Belisario (B) and DeRosa (D)) in Basilicata (Val d'Agri area) in two different years. 500 mg of ground dry seeds sample were extracted with 40 mL of 70% ethanol (pH 2.0 by HCOOH). Analyses of polyphenols were carried out by HPLC/DAD/MS. Flavonols and anthocyanins were separated by using a 150 × 3 mm, 4 μm, Synergy MAX-RP80A column (Phenomenex, USA). Hydroxycinnamic derivatives were separated by using a 150 × 4.6 mm, 5 μm, Luna RP18 column (Phenomenex, USA). Quantitative analyses were performed using five level calibration curves obtained with authentic standard.
Free radical scavenging activity was evaluated with the DPPH• assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams et al. *(6)* slightly modified.

Results and Discussion. The chromatographic profile of seed coats of the four analyzed varieties, recorded at 350 nm, is presented in Figure 1. According to literature [7], the chromatogram reveals the presents of flavonol derivatives in particular kaempferol glycosides.

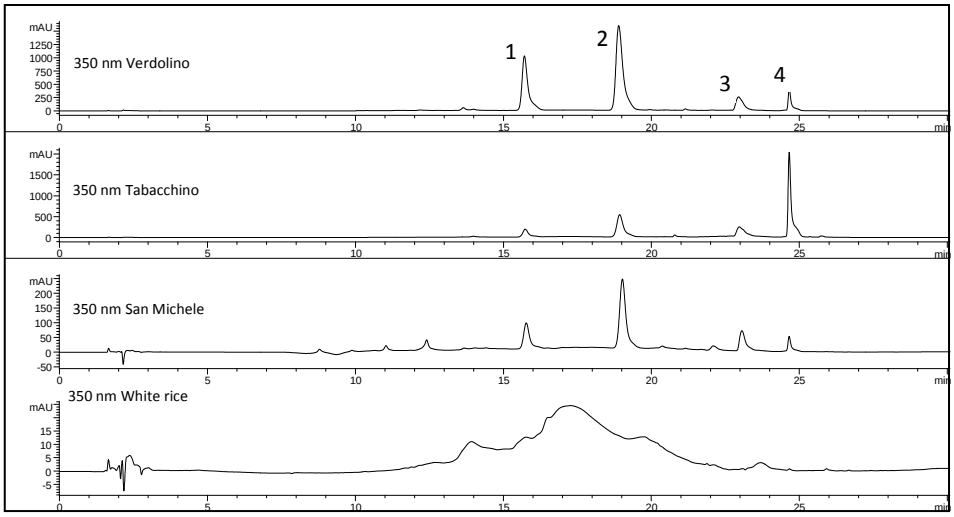
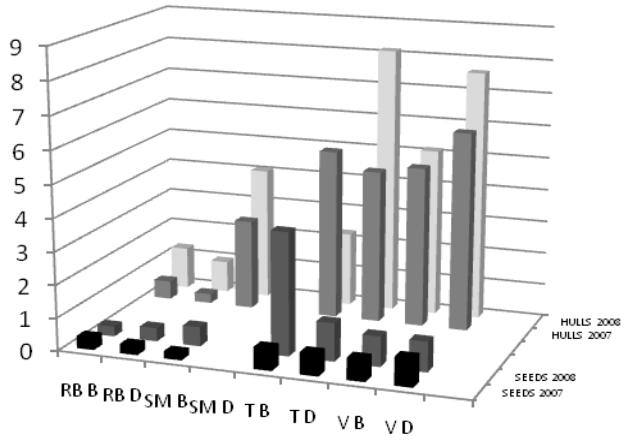


Figure 1. Identified flavonols: 1) kaempferol 3-*O*-xylosyl-glucoside; 2) kaempferol 3-*O*-glucoside; 3) kaempferol 3-*O*-(6"-*O*-malonylglucoside), 4) kaempferol

Cyanidin 3,5-*O*-diglucoside, pelargonidin 3,5-*O*-diglucoside; cyanidin 3-*O*-glucoside, pelargonidin 3-*O*-glucoside, cyanidin 3-*O*-(6"-*O*-malonylglucoside) and pelargonidin 3-*O*-(6"-*O*-malonylglucoside) have been found in the pigmented variety (SM). Among hydroxycinnamic derivatives, caffeic, ferulic and p-coumaric acid derivatives, have been found. Polyphenol content ranged from 0.353 (RB B) to 2.395 (T B) mg/g in seeds, harvested in 2007, and between 0.283 (RB B) and 2.484 (T B) in 2008, years. Figure 2 shows the polyphenol content, evaluated by HPLC/DAD, of the all analyzed varieties; we can notice that polyphenol content usually is higher in 2008 except for hulls of Tabacchino variety (Belisario). These data agree with weather conditions, since 2008 was a dry year and it is known that under stress conditions polyphenol metabolites increase [8].



The antioxidant capacity evaluated by DPPH test has been measured. The antioxidant activity is expressed as EC_{50} that indicates the mg of beans necessary to reduce the 50% of the activity of 1 mg of DPPH•; the lower the EC_{50} value, the higher the antioxidant activity of the sample. For example in 2008 samples, EC_{50} data range from 2.78 mg/mg (SM B) to 16.93 mg/mg (RB D).

Figure 2. Polyphenol content (mg/g) in 2007 and 2008 of bean varieties.

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